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# INFLUENCE OF TEMPERATURE, MOISTURE, AND SOIL REACTION ON THE DAMPING-OFF OF RED PINE SEEDLINGS BY PYTHIUM AND RHIZOCTONIA [1]

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## INTRODUCTION

The influence of environment on damping-off has been studied because of the great variability in the occurrence of the disease both in the same nursery from season to season and in different nurseries during the same season. There also may be great variation in different parts of the same nursery during the same season. A number of instances of this variability are recorded in the reports dealing with the fungi responsible.

The two primary causal agents in Wisconsin are *Pythium* and *Rhizoctonia* (*16*).[3] The literature relating to the activity of these organisms as they attack forest seedlings and various other plants is extensive. Much of that pertinent to this topic has been reviewed by Hartley (*7*), C. Roth (*15*), L. F. Roth,[4] and Ten Houten (*19*). The fungi causing damping-off of particular hosts may vary strikingly, occasionally within the same species, in their responses to environmental factors. This variability is doubtless influenced in turn by the usual increase of resistance by the seedlings with increase in age.

The purpose of the present study was to determine the influence of temperature, moisture, and soil reaction, respectively, on the damping-off of red pine (*Pinus resinosa* Ait.) in Wisconsin in regard to (1) germination of the seed and growth of the host, (2) vegetative growth of the two pathogens named, and (3) the interrelation of host and parasite for the development of disease.

## MATERIALS AND METHODS

The pathogenic fungi used most were cultures F–111–A of *Pythium irregulare* Buisman and F–5 of *Rhizoctonia solani* Kühn. Descriptions of these organisms are given elsewhere (*16*). In addition, laboratory studies were made with a culture of *Pythium debaryanum* (F–117) kindly supplied by W. F. Buchholtz and a particularly virulent strain of *Rhizoctonia* (F–118) isolated from seedlings in central Wisconsin.

The red pine seed used was from local stock collected recently and stored in tightly sealed bottles at about 40° F.

The soil was Plainfield sand secured from a 20-year-old stand of jack pine adjacent to the Griffith State Nursery at Wisconsin Rapids.

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[2] Acknowledgment is made to the Wisconsin Conservation Department for its cooperation and encouragement in these investigations. Assistance in many of the tests was furnished by the personnel of the Work Projects Administration, Official Project No. 65-1-53-2349. The writers are indebted to Eugene Herrling for assistance in preparing the illustrations.

[3] Italic numbers in parentheses refer to Literature Cited, p. 284.

[4] ROTH, L. F. THE INFLUENCE OF ENVIRONMENTAL FACTORS UPON THE DAMPING-OFF DISEASE OF CONIFER SEEDLINGS CAUSED BY PYTHIUM AND RHIZOCTONIA. 1940. [Unpublished doctor's thesis. Copy on file, University of Wisconsin library, Madison, Wis.]

This soil was used without further sterilization because it was practically or entirely free from *Pythium* and *Rhizoctonia*. Its reaction was pH 5.5. An analysis provided by S. A. Wilde showed the following approximate composition: Silt and clay, 10.5 percent; base exchange, 5.5 milliequivalents per 100 gm.; total nitrogen, 0.1 percent; organic matter, 3.7 percent; available phosphate, 35 pounds per acre; available potash, 150 pounds per acre; and replaceable calcium, 2.9 milliequivalents per 100 g.m.

The soil showed no tendency to puddle or cake under varying moisture conditions.

Germination was hastened from about 4 days to a month, according to the temperature, by keeping the seed 4 days on moist filter paper at 28° C. This treatment saved much time and had no apparent ill effects on the seedlings. The period between germination and emergence remained unchanged.

The more common methods for inoculating soil were found less satisfactory than the following: About 100 clean red pine seedlings. from 1 to 3 weeks old, were cut at the ground level and chopped into pieces 1 to 5 mm. long. In each of the 90-mm. Petri dishes used in growing the inoculum, 50 to 100 of these pieces of fresh tissue were mixed with water agar just before solidification. The plates were inoculated at the center and incubated at laboratory temperature. The damping-off fungi covered the agar before any contaminants introduced on the chopped seedlings became established. The agar mycelial mat was cut into 2-mm. squares. One Petri dish served for one 6-inch round container in which 100 pine seeds were planted. Smaller containers received proportionately less inoculum and seed. The inoculum was distributed uniformly over the soil surface, the seed was planted at the same level, and the whole was covered with about three-sixteenths inch of soil.

The amount of disease was based upon the proportion of individual seedlings destroyed. After the seed came up, counts were made every 3 days for about 4 weeks, by which time the seedlings had developed resistance. After the final count, records were prepared of total postemergence damping-off, survival, and total emergence. Parallel, uninoculated controls were always provided. The percentage of emergence was calculated from that in the controls. The preemergence damping-off was calculated by subtracting the percentage of total emergence in the inoculated soil from that of emergence in the controls.

The identity of the fungi causing the disease was determined in all cases. Representative diseased seedlings up to a dozen, selected according to their suitability for plating, were culturally examined on water agar. In every case only the culture introduced was recovered.

Measurements of host development as determined by height of above-ground parts and weight of entire seedlings, wet and dry, were inadequate. Root length alone varied uniformly in Plainfield sand in relation to temperature and acidity. Consequently, the mean root length of 50 seedlings taken at random and washed out of the control containers has been used as the measure of host development.

## TEMPERATURE

### TEMPERATURE AND THE PATHOGENS IN CULTURE

The influence of temperature upon growth of two isolates of *Pythium* and two of *Rhizoctonia* was examined. For each isolate four Petri dishes of potato-dextrose agar, pH 5.8, were seeded in the center with a 2-mm. culture-bearing disk of similar agar. Four dishes were wrapped in wax paper and incubated at each of nine temperatures, ranging in 4° intervals from 4° to 36° C. Of measurements made at 12-hour intervals after inoculation, those made at 36 hours showed the most satisfactory range and were best suited to graphic presentation. The mean values for the four colony diameters at each temperature in one of three separate trials are shown in figure 1.

Growth of the four strains was best at 28° C. and good between 18° to 34°. Throughout this range up to about 34° *Pythium* grew more

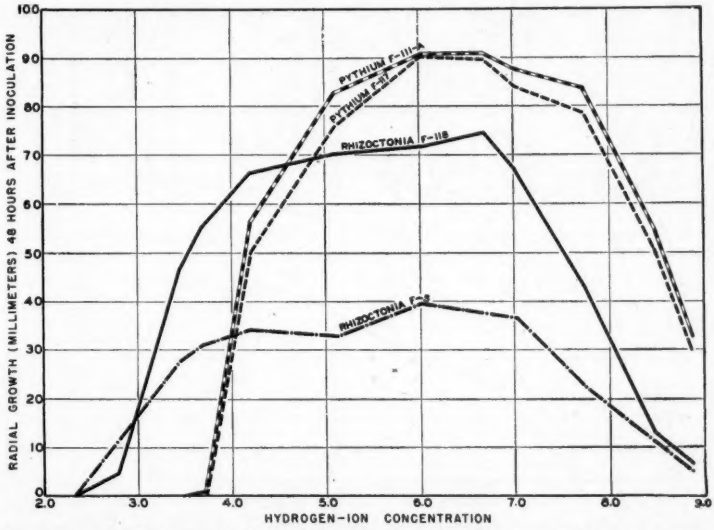


FIGURE 1.—Radial growth in millimeters of four damping-off fungi on potato, dextrose agar incubated at various maintained temperatures.

rapidly than *Rhizoctonia*. From 12° to 32° growth of the 2 strains of *Rhizoctonia* was similar, and that for the two species of *Pythium* was similar. However, at 36° one strain of *Rhizoctonia* showed little decline while the other and the two of *Pythium* were declining rapidly. Although the good vegetative growth of both groups between 18° and 32° suggested a preference for warm temperatures, the effective pathogenicity of *Pythium* extended well down into the cooler temperatures. While growth was slower, much more time was available at cooler temperatures because of the reduced growth rate of the seedlings and their consequently prolonged susceptibility.

TEMPERATURE AND THE GERMINATION OF RED PINE SEED

The germination of red pine seed was examined in the larger experiments dealing with pathogenicity. In this case the controls, which ran parallel to seed in inoculated soil, were used. The tests were made in Wisconsin soil-temperature tanks (*11*) with temperatures ranging from 12° to 33° C. at 3° intervals. Two tanks were placed in each of 4 constant-temperature houses, thus: 12° and 15° tanks were in a 16° house, 18° and 21° tanks were in a 20° house, 24° and 27° tanks were in a 24° house, and 30° and 33° tanks were in a 28° house.

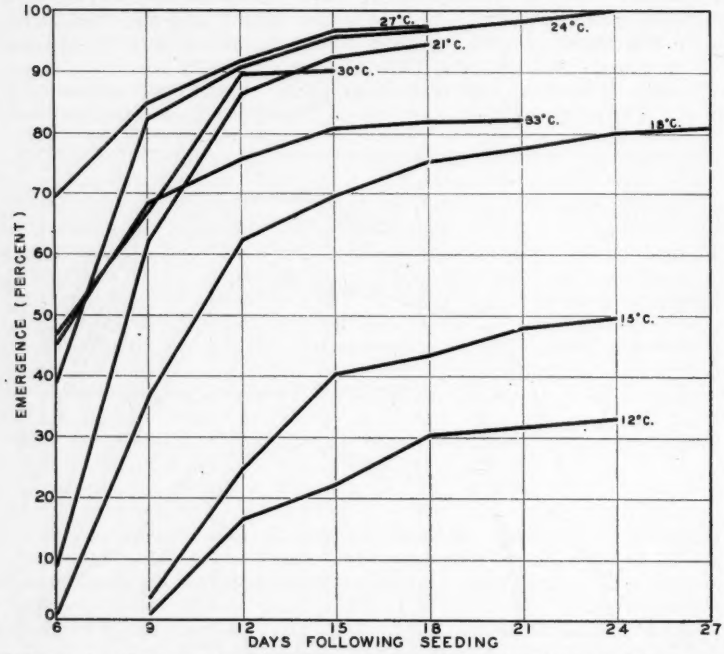


FIGURE 2.—Rate and amount of emergence of red pine seed at different soil temperatures (C.).

Each tank accommodated 8 cylindrical cans 6 inches in diameter and 6 inches deep. Out of every 8 cans, 3 were inoculated with *Pythium* and 3 with *Rhizoctonia*, while 2 were uninoculated controls. The cans were placed in the tanks in a random manner. Germination was uniform, except at 12°, where the variation was not significant.

Throughout these temperature studies the soil was commonly maintained at 60 percent of its moisture-holding capacity against free drainage by frequent weighing and by adding water uniformly to the surface when necessary to restore the original weight. The customary central watering tubes served primarily as supports for covers of nonmoistureproof cellophane. These covers reduced surface evaporation, by their nonmoistureproof character prevented complicating drip from water of condensation, and eliminated contamination between cans.

The temperature strongly influenced both the rate and the amount of emergence. The best emergence was secured at 24° C., where 89 percent of seed planted came up. For easy comparison of the effects of temperature, the best emergence is considered as 100 percent and the percentage emergence at other temperatures determined accordingly. The results of four different experiments have been averaged for the curves in figure 2. Emergence was poor at 12° and 15°, good at 18° and 33°, and excellent from 21° to 30°.

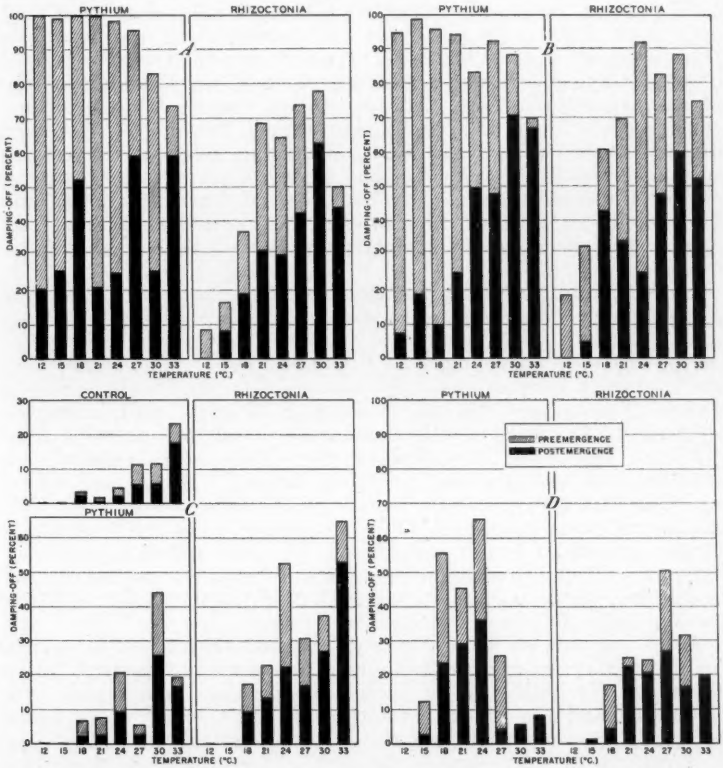


FIGURE 3.—Total preemergence and postemergence damping-off of red pine seedlings in Plainfield sand caused by *Pythium* and *Rhizoctonia* at different temperatures: *A*, Steamed, moisture at 60 percent of moisture-holding capacity, pH 6.4; *B*, same as *A* except for pH, 4.8; *C*, unheated forest soil, moisture at 60 percent of moisture-holding capacity, pH 5.5; *D*, steamed, moisture at 40 percent of moisture-holding capacity, pH 5.5.

## TEMPERATURE AND DAMPING-OFF

Warm temperatures are generally considered (*10*, *7*) to favor either the amount of damping-off or its rate of appearance on conifers. C. Roth (*15*) found damping-off fungi most destructive at intermediate temperatures while Gravatt (*5*) observed that *Rhizoctonia* was most

aggressive at low temperatures. Such differences are not surprising in view of the variability of the different fungi and the different hosts involved. Similarly, other varying factors of the environment influence the temperature response. On hosts other than forest seedlings various workers have reported great variation in the temperature relations of *Pythium* and *Rhizoctonia* (as reviewed by L. F. Roth (1940) [5]).

The effect of controlled temperature on damping-off by both *Pythium* and *Rhizoctonia* was examined in 11 series of soil-temperature studies between 1937 and 1940. When free from biological competition, *Pythium* commonly killed most of the seedlings at 12° C. and only about half of them at 33°, with intermediate results between these temperatures. *Rhizoctonia* started with relatively few damped-off at 12° but approached a maximum of between 50 and 98 percent at warmer temperatures. This maximum varied with other conditions but was fairly well maintained between 20° and 30°. A sharp decline followed at 33°. Thus both fungi were active at the temperatures favorable for host development, *Pythium* having some advantage at cooler temperatures, *Rhizoctonia* some at warmer temperatures. The details on most of these studies are omitted because of their volume and because the summary on those reported in detail is representative.

The effect of temperature was modified, particularly by soil moisture and reaction. The influence of these factors is graphically summarized in figure 3, which shows the results in four different tank series set up with several varying lots of Plainfield sand. These series have been selected because they represent certain important variations deserving attention. The soil in series *A*, *B*, and *C* held moisture at 60 percent of its moisture-holding capacity and that in series *D*, 40 percent. The soil in *C* and *D* was forest soil secured near the nursery, and that in *A* and *B* was soil from the nursery. *A* had been treated with lime and *B* with sulphur 1 and 3 years earlier, respectively. The reaction of *A* was pH 6.4; *B*, 4.8; *C* and *D*, 5.5. The soil in *A*, *B*, and *D* was autoclaved for 10 minutes at 15 pounds pressure to kill any damping-off fungi that might be present. There was no damping-off in the controls of the heated soil. That in the unheated resembled the damping-off caused by *Fusarium* (C. Roth, *15*).

Total damping-off caused by *Pythium* and *Rhizoctonia* at each of the eight temperatures for the four series is shown by vertical bars in figure 3. The solid part of each bar represents damping-off after emergence and the shaded part, preemergence loss. As explained before, for easy comparison of the effect of damping-off, independent of variations in emergence at different temperatures, the emergence in the controls has been taken as 100 percent. The isolate of *Pythium* was more virulent than that of *Rhizoctonia*. Consequently, where other factors did not influence the relationship, the attack by *Pythium* in these particular experiments was more effective than that by *Rhizoctonia*. In other studies this difference between *Pythium* and *Rhizoctonia* was not apparent.

The amount of seedling growth at different temperatures was recorded. At the close of each of these 4 temperature experiments, the seedlings from the control cans at each temperature were washed free of soil. From each can 25 were selected at random and the root

[5] See footnote 4, p. 265.

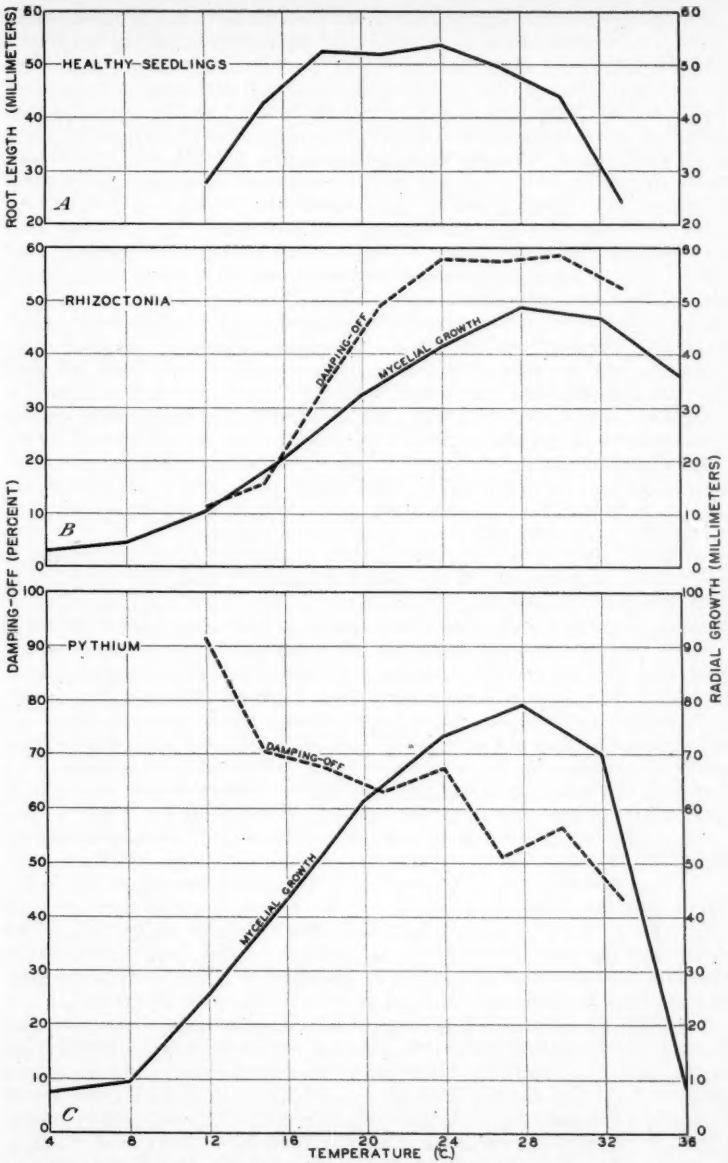


FIGURE 4.—The influence of temperature on: *A*, Root growth of healthy seedlings; *B*, growth of *Rhizoctonia* in culture and the percent of damping-off; *C*, growth of *Pythium* in culture and the percent of damping-off.

lengths measured. Since there were 2 cans in each of the 4 experiments, each point on the curves in figure 4 represents the average of 200 seedlings.

The responses to different temperatures of the vegetative growth by host and parasite and of the damping-off when the two are acting together are summarized in figure 4. The different items are placed in juxtaposition for easy comparison. The growth of the red pine seedlings as indicated by root length is given in *A*. This curve shows that red pine developed well between about 15° and 30° C. At 30° and 33°, the warm temperature induced spindling growth, weak hypocotyls, and pale color, as well as poor root development. Deficient growth appeared also at temperatures below 15°, particularly in soil having a low moisture content. The size of mycelial mats in culture and the percentage of damping-off by *Rhizoctonia* are shown in *B* and those of *Pythium* in *C*. The amount of dampıng-off is the average from several tank series in which conditions were favorable for the development of damping-off.

Where moisture conditions were favorable and the pathogen was not competing strongly with other fungi (fig. 3, *A* and *B*), total damping-off caused by *Pythium* decreased from a broad maximum lying between 12° and 21° C. to a minimum at 33°. The opposing curves of fungus growth and damping-off (fig. 4, *C*) at cooler temperatures are quite striking.

When *Pythium* was exposed to the competition of the abundant microflora and fauna of the nonautoclaved forest soil (fig. 3, *C*) but was under favorable conditions of soil moisture, the effect of temperature was quite different. No damping-off occurred at either 12° or 15°. Above 15° damping-off increased to a maximum at 30° and then fell off at 33°. Platings of damped-off seedlings from all *Pythium* cans of this series were made. Only at 24° and 30° C. (fig. 3, *C*) did the number of isolates of *Pythium* exceed that of the unidentified fungus resembling *Fusarium* found in the controls. These results emphasized the importance of biological competition as a factor influencing damping-off. A study of this aspect, however, is beyond the scope of the present paper.

Soils of pH 4.8 showed somewhat less postemergence damping-off by *Pythium* at the cooler temperatures than soils with pH 6.4.

At cool temperatures *Pythium* was most easily stimulated by other conditions favorable to aggressive growth but was also most quickly inhibited by unfavorable conditions. Since various competıng microorganisms are active in nature and since the moisture of the soil is often low, *Pythium* development in the nursery is likely to be greatest at temperatures between 18° and 24° C.

Greatest *Rhizoctonia* damping-off in all series (fig. 3) occurred at moderately warm temperatures. As temperature became unfavorable for parasite development, damping-off decreased rapidly to a minimum near 12° C. The similarity of curves in figure 4 for fungus growth and damping-off is striking. As was the case with *Pythium*, differences in pH of 4.8 and 6.4 had little if any influence on the trend of total damping-off. The magnitude of the *Rhizoctonia* loss, however, was significantly greater in the more acid soil. Biological or soil-moisture factors in these studies failed to influence *Rhizoctonia* to the degree that they affected *Pythium*. The *Rhizoctonia* attack at 33° in series

*C* was approximately that in either *A* or *B*, suggesting perhaps that it was inhibited by warm temperature less than its competitors. At cooler temperatures, however, competition seemed to diminish the attack. The height of the postemergence bars in figure 3, *D*, indicates that *Rhizoctonia* damping-off in dry soils did not respond to temperature between 18° and 33° with the variability shown in wet soil. Below 18° in both dry soil and unheated soil, damping-off by both fungi fell off to insignificance.

Considering the four series as a whole, the general trend of postemergence damping-off for both fungi was up with rising temperature. Such a response probably accounts for the established belief that damping-off is most severe at warmer temperatures.

However, in the graphs for *Pythium*, postemergence damping-off appears an inadequate measure of actual injury. At the cooler temperatures in moist soil and in the absence of competition (fig. 3, *A* and *B*), losses through preemergence damping-off appear excessive and unrelated to the rate of vegetative growth of the fungus (fig. 4, *C*). It is apparent, however, that at 12° C., the lowest temperature allowing germination and measurement of damping-off (figs. 2, 4, *C*), the fungus grew rapidly enough (fig. 4, *C*) to allow reasonably prompt dissemination throughout the container. This high loss appears, therefore, to be correlated with slow germination and seedling development (fig. 4, *A*). At cool temperatures in dry soil (fig. 3, *D*) and with competition (fig. 3, *C*) the proportion of preemergence to postemergence damping-off by *Pythium* diminishes to not much above 1 : 1.

With *Rhizoctonia*, however, the proportion of postemergence to preemergence damping-off appears relatively constant at all except warm temperatures and is roughly 1 : 1. A correlation between temperature, preemergence loss, and the vegetative growth of the fungus is evident. At cooler temperatures, slow fungus growth seems a factor limiting disease development. Ramification throughout the soil or extension beyond the point of original infection was so retarded that no rise appears in the damping-off curve even though slow emergence (fig. 2) and development of the seedlings exposed them for a longer time to fungus attack (fig. 4, *A*). With rising temperatures favoring mycelial growth the curve for damping-off also rises (fig. 4, *B*). The decline in *Rhizoctonia* damping-off above 30° C. appears similarly correlated with poor fungus development.

## MOISTURE

### SOIL MOISTURE

The importance of soil moisture for damping-off is accepted generally, but there is considerable divergence concerning details (*7*, *20*, *15*, *6*). The subject has been reviewed by L. F. Roth.[6] This lack of agreement has doubtless arisen from various conditions other than moisture. To clarify these influences, several experiments have been made testing the effect of varying moisture in Wisconsin nursery soil.

The maintenance of the desired moisture content of the soil, particularly at the all-important top half inch, presented various problems. Preliminary experiments were conducted with 2-quart crocks in which the moisture content was regulated by the weighing

[6] See footnote 4, p. 265.

method. While the results were in general agreement with those given later, so much variability arose from inadequate moisture control that the details have been omitted.

The method used depended on the uniform capillary rise of water in the Plainfield sand. Thirty-six galvanized-iron 4-inch cylinders, each having one end covered with galvanized fly screen, were prepared. The cylinders varied in height, sets of six being 4.5, 6, 7.5, 9, 10.5, and 12 inches, respectively.

Two watertight, flat-bottomed pans 14 by 28 inches with 3-inch rims accommodated the cylinders. Each cylinder, with a sheet of filter paper over its screen bottom, was completely filled with Plainfield sand, pH 5.5. The soil was packed by dropping the cylinders 1 inch 15 times. After the 10.5- and 12-inch cylinders had been dropped twice, they were again filled and dropped the remaining 13 times

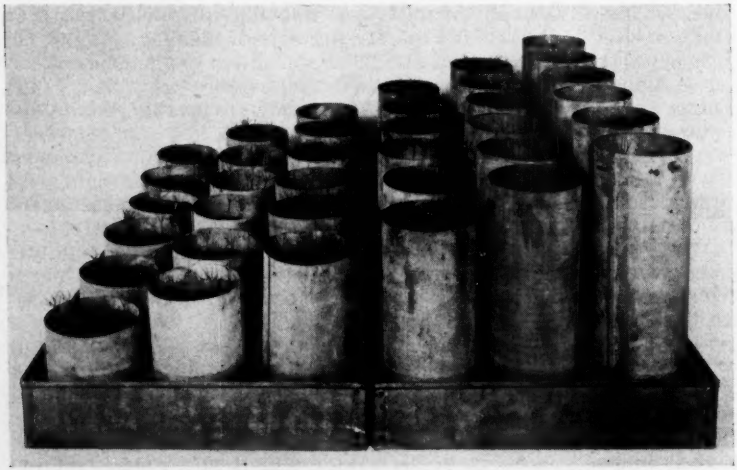

FIGURE 5.—Soil moisture equipment in which the moisture of the surface soil was regulated by the height of the capillary column from the water in the pans. A nonmoistureproof cellophane cover was commonly placed over each cylinder.

Cylinders of the 2 intermediate heights were dropped once before they were refilled while the 2 shorter groups were dropped 15 times without refilling. The packed soil in all cylinders came within about an inch of the top. The cylinders were arranged in graduated order in the pans in which one-half inch of freely circulating water was maintained. The temperature during these moisture studies fluctuated around the favorable 24° C. After 25 seeds had been planted and the soil in the top of each soil cylinder had been inoculated according to the method described for the temperature studies, a piece of nonmoistureproof cellophane was fixed over each cylinder. A cheesecloth cover was placed over the entire unit to prevent excessive light and evaporation. The relative humidity of the greenhouse air varied around 50 percent. An experiment in progress is shown in figure 5.

The efficiency of this method for controlling the moisture content in the surface layers of the soil used is shown in figure 6, *A*, where the

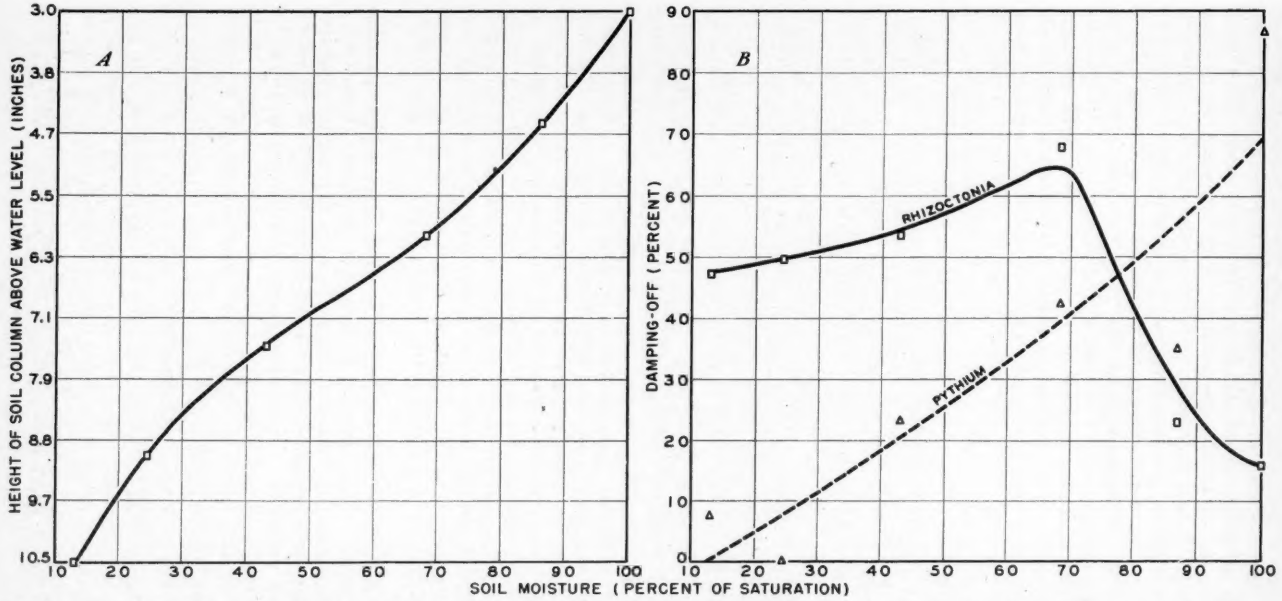


FIGURE 6.—Soil moisture and damping-off: *A*, Moisture content of the upper one-half inch of soil, showing efficiency of soil columns of graduated heights for regulating surface moisture; *B*, damping-off of red pine in virgin Plainfield sand, inoculated with *Pythium* and *Rhizoctonia* under the moisture conditions shown in *A*.

moisture content of the upper one-half inch of soil is plotted against the height of the soil column in inches above the water level. The curve shows a progressive increase in soil moisture, from 13 percent to 100 percent saturation, corresponding with decrease in column height. Since the minimum moisture for germination lies between 30 and 35 percent, it was necessary at the beginning to add some water at the surface of the two tallest series of cylinders. After germination, but before emergence, the moisture level was allowed to assume normal value.

The amount of moisture for host development varied with columns of different height. Average root lengths of seedlings washed from uninoculated control containers in a representative experiment were as follows:

| | |
|---|---|
| Root length (millimeters) | 57, 48, 46, 43, 43, and 38 |
| Soil moisture (percent) | 13, 25, 43, 68, 86, and 100 |

In soil with 13-percent moisture the root elongation was rapid. Growth was straight down with some lateral branching. The roots had a relatively dark color, suggesting early suberization, which, if anything, probably increased their resistance to damping-off. In the saturated soil, root growth was very slow and tortuous, branching was abundant, and the main roots penetrated little below the original depth of the seed. The roots remained succulent and unsuberized for a month and thus probably maintained susceptibility. In the four soils with intermediate moistures, root lengths and appearance were also intermediate.

In each soil moisture series two cylinders of each height were inoculated with *Pythium* and two with *Rhizoctonia*. The remaining two were left uninoculated as controls. Each cylinder was planted with 25 red pine seeds. The experiments were repeated four times. While all the findings were essentially similar, some variability was observed. The results of a representative series are given in figure 6, *B*, where the damping-off, expressed as percentage of emergence in the controls, is plotted against soil moisture. In this case 87 percent of the seedlings damped-off in the *Pythium* inoculations in saturated soil. However, probably because of error, only about 35 percent damping-off appeared in the *Pythium* inoculations at 86 percent moisture. Such a drop at this point was not found in three other experiments.

The curve of *Pythium* damping-off in figure 6, *B*, rises steadily with increasing moisture. The curve for *Rhizoctonia* damping-off rises very gradually as soil moisture increases from 13 to 68 percent of capacity. After this point there is a marked decline of damping-off to a minimum at 100 percent moisture. The entire range studied below about 80 percent may be considered favorable to *Rhizoctonia* damping-off while soil moisture greater than this appears to inhibit the attack, provided atmospheric humidity is relatively low.

### AIR HUMIDITY

The importance of the relative humidity of the air appeared in one soil moisture experiment when the nonmoistureproof covers were replaced by moistureproof cellophane placed tightly over the tops of the cylinders. In those having above 70 percent soil moisture, infection increased to 100 percent damping-off at saturation. All cylinders

with soil moisture above 60 percent showed abundant aerial mycelium. At high humidities the seedling cotyledons were commonly attacked.

The influence of air humidity as well as soil moisture upon damping-off was studied in view of this response. Seventy-two 4-inch pots were filled with clean, untreated Plainfield sand, pH 5.5, planted with 35 red pine seeds, and divided into 3 equal lots. One lot was inoculated with *Pythium*, one with *Rhizoctonia*, and the third was left uninoculated. Since the soil was not sterilized, a small amount of damping-off occurred in the controls, particularly those at 90 percent moisture. Twelve of each inoculation series were adjusted to approximately 90 percent saturation and the remaining 12 to 35 percent. Six of the pots of each moisture group were placed in a cheesecloth-covered cage having a relative humidity of approximately 50 percent. The other 6 pots were kept in a glass humidity chamber equipped with a spray and a circulation fan. Greenhouse temperatures in both cases were about 24° C. Only postemergence damping-off was considered in interpreting the results. The data are given in table 1 without correction for germination. As in the preceding experiments, soil moisture greatly influenced damping-off. The loss in pots inoculated with *Pythium* and containing 90 percent moisture was 90 percent or greater while less than 1 percent damped-off in the dry pots. Air humidity had little influence on the amount of damping-off in pots inoculated with *Pythium*. However, high air humidity increased the loss caused by *Rhizoctonia*, irrespective of soil moisture. As in the studies on soil moisture, *Rhizoctonia* losses were greater in dryer soils.

TABLE 1.—*Influence of soil moisture and air humidity upon damping-off by Pythium and Rhizoctonia*

| Fungus inoculated | Soil moisture | Approximate relative humidity | Emergence | Postemergence damping-off | |
|---|---|---|---|---|---|
| | *Percent* | *Percent* | *Number* [2] | *Number* | *Percent* |
| Control | 90 | 98 | 147 | 20 | 14 |
| | | 50 | 163 | 19 | 12 |
| | 35 | 98 | 182 | 9 | 5 |
| | | 50 | 156 | 0 | 0 |
| *Pythium* | 90 | 98 | 140 | 138 | 99 |
| | | 50 | 108 | 97 | 90 |
| | 35 | 98 | 188 | 1 | 0 |
| | | 50 | 155 | 2 | 1 |
| *Rhizoctonia* | 90 | 98 | 94 | 63 | 67 |
| | | 50 | 91 | 39 | 43 |
| | 35 | 98 | 106 | 89 | 84 |
| | | 50 | 109 | 61 | 56 |

[1] Approximate percent of moisture-holding capacity. The moisture content was maintained by subirrigation.
[2] 210 seeds were planted in the 6 pots of each treatment.

## ACIDITY

The adjustment of soil reaction has been commonly and often successfully used to control damping-off. Thus the influence of acidity of the medium upon growth of the fungi in culture, as well as the effect of soil reaction upon both the host and disease development, becomes of especial interest.

The success of acid treatments in disease control, considered with the observation of Gifford (*3*) that applications of lime and wood

ashes increased damping-off, has led to the general conclusion that the disease is favored by neutral or alkaline soils. Wilde (*21*) considers the most desirable reaction for nursery soil to be between pH 5.0 and 6.0. He has stated that "* * * a reaction of soil higher than pH 6.5 is highly undesirable in the nursery since it provides the optimum condition for the development of damping off fungi."

Studies on the distribution in sandy Wisconsin soils of the two fungi considered have shown that *Pythium* predominates (*16*) in soil more alkaline than about pH 5.8 and *Rhizoctonia* in soil more acid. Likewise, in figure 3 it appears that the relative activity of these two fungi was altered by a change in reaction. This relationship was not found on soils of calcareous origin. On heavy soils, even as acid as pH 4.2, *Pythium* was found abundant. This observation is in accord with the opinion of Hartley (*7*) and of Gäumann (*2*) that humus content influences the abundance of *Pythium*.

The above observations suggest that damping-off may not be closely limited to slightly acid or neutral soils but may, in fact, be favored by moderately to highly acid soils. They are in accord with the suggestion that part of the effectiveness of acid treatment comes from partial soil surface disinfection by the acid.

### ACIDITY AND THE PATHOGENS

The influence of reaction on the growth of *Pythium* and *Rhizoctonia* in culture has been the subject of frequent studies and divergent observations. Various investigators (*9*, *8*, *15*, *1*) report the minimum pH for growth of *Pythium* to be between pH 3.1 and 4.6. Their results indicate the optimum to be between 3.43 and 8.3 and the upper limit between 7.2 and 9.6. *Rhizoctonia* is reported (*15*, *4*, *12*, *13*, *18*) as limited in growth at pH values between 2.4 and 3.8. Optima are given by the several workers as ranging from pH 4.5 to 7 and upper limits from 6.7 to 9.1. Because of this variation it seemed necessary to determine these acidity relations for the fungi already studied intensively.

Potato-dextrose agar was used in these experiments. The medium was autoclaved in 200-ml. lots. Before it had solidified, 20 ml. of M/3 sterilized phosphates ($H_3PO_4$, $NaH_2PO_4$, $Na_2HPO_4$, and $Na_3PO_4$) in suitable combinations and 0.1 ml. of sterile concentrated sulfuric acid were added to each 200 ml. of medium. The flasks were shaken thoroughly and the agar poured into 13 plates, each containing approximately 15 cc. of agar.

Each of triplicate plates at each reaction was seeded at the center. A 2-mm. disk was used from the advancing margin of a young culture. Four isolates, occupying 12 plates, were studied: *Pythium* F–111–A and F–117 and *Rhizoctonia* F–118 and F–5. At the time of transfer a thirteenth similar plate was heated just to melting and its reaction determined with a glass electrode corrected for temperature. The diameter of each of the colonies was measured every 12 hours, and the vertical and horizontal measurements of each were averaged for a given reading. All cultures were incubated at 24° C.

The results of one experiment in which the 48-hour measurements were plotted are given in figure 7.

Growth of *Pythium* cultures extended from pH 3.7 to about 9 and was most rapid between 5.0 and 8.0. *Rhizoctonia* grew between pH

2.4 and about 9 and most rapidly between 3.5 and 7. Excellent growth for both fungi occurred around pH 6. On relatively acid media, at pH 3 to 4, *Rhizoctonia* grew well, but *Pythium* grew little if at all. However, in media between about 8 and 8.5 *Pythium* grew well while *Rhizoctonia* grew poorly. Growth rate of the two species of *Pythium* was almost identical throughout the range, whereas the rate of growth of the two strains of *Rhizoctonia* differed greatly. Under optimum conditions *Pythium* was inherently faster growing than *Rhizoctonia*.

Only hydrogen-ion concentration was controlled and measured in these investigations. However, as is well known (*14*), very acid media may have a strongly positive Eh and strongly alkaline media a negative Eh. Furthermore, when growth had once started, the metabolic

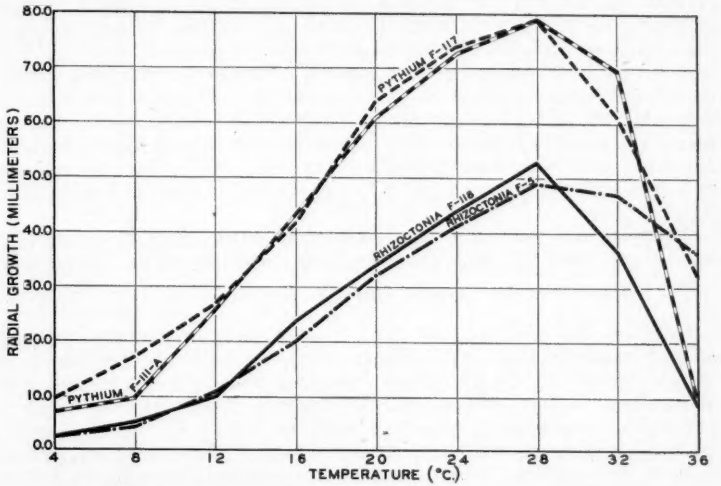


FIGURE 7.—Influence of hydrogen-ion concentration on growth of four damping-off fungi on potato-dextrose agar, adjusted with phosphates over a pH range of 2.3 to 8.9. The values given represent mean measurements in millimeters of two diameters of triplicate colonies at 24° C. 48 hours after inoculation.

activity of the fungi and atmospheric $CO_2$ probably influenced the acidity, the oxidation-reduction potential, and various other properties of the medium, which might in turn have affected the growth of the cultures.

### ACIDITY AND THE HOST

The influence of soil reaction on host development was observed in the controls of experiments designed for study of the influence of pH on damping-off.

The reaction of the Plainfield sand was adjusted by treating one portion with hydrated lime and another with 3 percent sulphuric acid. The soil was placed out of doors during much of November and December, where it frequently froze and thawed. In the greenhouse the acid and alkaline portions were mixed with untreated soil in various ratios to give a graded series of soil reactions. While this

method has some obvious objections, it was used because of the desire to maintain natural conditions as far as possible with respect to other factors involved. After standing for 2 weeks and receiving abundant watering, the different lots of soil were placed in 4-inch pots, autoclaved, and their acidity measured with a glass electrode. A series of nine lots having, respectively, the following reactions was chosen: pH 4.4, 4.9, 5.3, 6.1, 6.6, 7.2, 7.8, 7.9, 8.4. Triplicate pots at each reaction were planted with 25 red pine seeds each and kept as controls. These served for examining the effect of soil reaction on the growth of the host plant. A similar set was inoculated with *Pythium* and a third set with *Rhizoctonia*, as reported in a later section. All the pots were watered, when necessary, with a sprinkling can, and thus the surface had a moisture content varying from about 30 to 100 percent. After 3 weeks under a cheesecloth cover in a 24° C. greenhouse, all seedlings were washed out of the controls, 50 were taken at random from each lot and their root lengths measured.

The optimum range for root growth extended from about pH 4.7 to 6.0 as shown in figure 8, *A*. Growth fell off rapidly at the acid side, but the limit for growth was outside the range used. On the alkaline side growth was relatively poor between about pH 6.0 and 8.0. No top injury was observed in any case, but there was reduced germination (30 percent) at pH 8.5. A second experiment gave comparable data.

These results were in general agreement with those of Wilde (*21*), who found that red pine grew well over a range of pH 5.0 to 6.0.

### ACIDITY AND DAMPING-OFF

Detailed investigations of the influence of acidity upon damping-off were made by Hartley (*7*), Jackson (*9*), and C. Roth (*15*), who have reviewed much of the literature. Jackson observed that damping-off of seedlings with *Pythium* and *Rhizoctonia* increased from none at pH 2.5 to a maximum near the neutral point. C. Roth observed the optimum soil reaction for attack by both *Pythium* and *Rhizoctonia* to lie between pH 6.7 and 7.0 with a broad range favorable to growth between pH 5.5 and 8.3. However, with acidity increasing below pH 7.0 the attack by *Pythium* was strongly retarded and was diminished to 0 at pH 4.5. *Rhizoctonia*, on the other hand, caused some damping-off at pH 3.7. Damping-off in general decreased, as Hartley (*7*) observed, with increasing acidity from pH 8.5 to 5.0. He found that, in general, damping-off was not serious in nursery soil more acid than pH 6.0.

The results with Wisconsin materials were secured through the experiment described in the preceding section. Two separate trials gave results which were similar, except that the amount of damping-off was somewhat greater, particularly with *Rhizoctonia*, in the first trial than in the second. The results were averaged for figure 8, *B* and *C*.

A broad reaction range, very favorable for attack by *Pythium*, extended from about pH 5.2 to beyond 8.5. Below 5.2 the amount of damping-off fell rapidly. On the other hand, for *Rhizoctonia* damping-off there appeared a broad range not too favorable for attack between pH 5.2 and 7.8. At the acid end damping-off increased rapidly to a maximum at pH 4.4. The upturn on the alkaline side may arise from the very poor growth of the seedlings near pH 8.2.

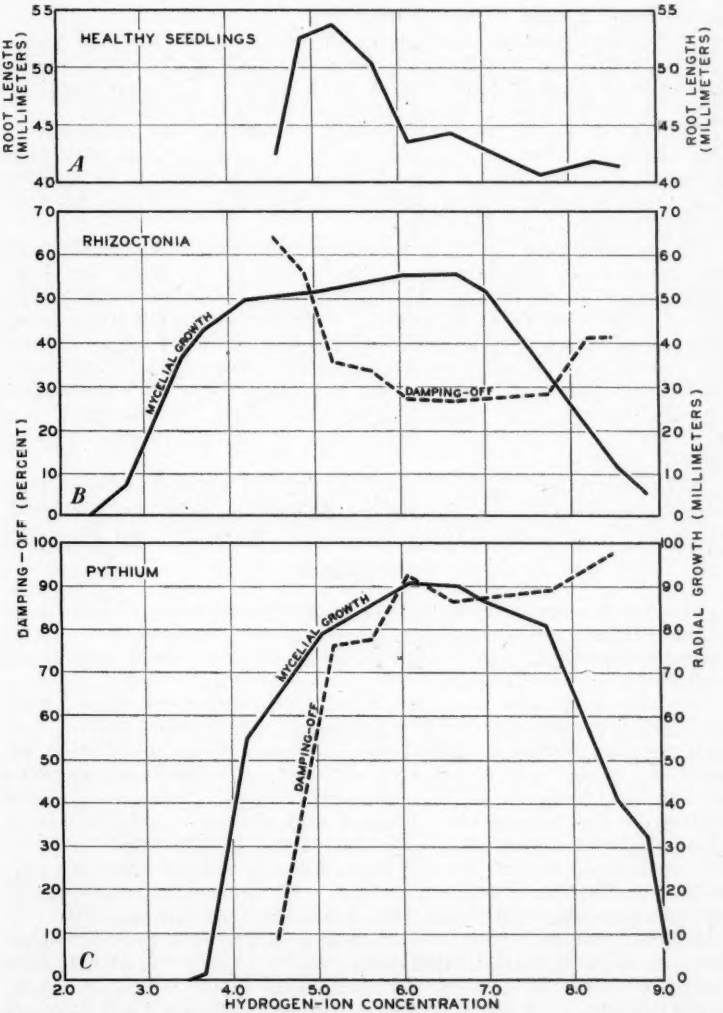


FIGURE 8.—The influence of hydrogen-ion concentration upon: *A*, root length of seedlings; *B*, growth of *Rhizoctonia* in culture and damping-off in soil; *C*, growth of *Pythium* in culture and damping-off in soil.

Increased damping-off by both fungi in soils more alkaline than pH 7.8 appeared correlated with poor host development. Though total damping-off by *Rhizoctonia* was lower than that caused by *Pythium* throughout the range more alkaline than pH 5.2, reaction at no point became a limiting factor.

In juxtaposition to these curves for damping-off (fig. 8, *B* and *C*) are the curves for growth of the fungi in culture at the corresponding reactions. These growth curves were secured by averaging those given in figure 7 for *Pythium* and *Rhizoctonia*, respectively.

The curves representing the growth of *Pythium*, of host plant, and the amount of *Pythium* damping-off (fig. 8, *C* and *A*) roughly correspond in the acid ranges. On the alkaline side *Pythium* damping-off rose while mycelial growth declined. This effect may have been due to reduced growth of the host.

The relation between the curves of damping-off and mycelial growth for *Rhizoctonia* differs markedly from that for *Pythium* (fig. 8, *B*). Reduced damping-off by *Rhizoctonia* and good mycelial growth occurred between about 5.5 and 7.5. Except on the alkaline end, the damping-off data were all taken within the pH range very favorable for growth of *Rhizoctonia*. Damping-off increased with increasing acidity from about pH 6.0. At pH 4.5, where there was still excellent mycelial growth, host development was limited. Consequently, as with *Pythium*, damping-off increased near pH 8.2 though mycelial growth declined.

Soil reaction had no effect upon the ratio of preemergence to postemergence damping-off in these experiments. Wherever damping-off occurred, the loss was approximately equal between these two types.

## DISCUSSION

The distinct responses of *Pythium* and *Rhizoctonia* to the influence of temperature, moisture, and soil reaction indicate that damping-off, caused independently by these fungi, may be most wisely considered and treated as two diseases.

Contrary to general concepts, three conditions merit special mention: (1) Damping-off need not necessarily, as its name implies, be a high-moisture disease. *Rhizoctonia* is favored by intermediate moistures and often operates in very dry soil. Excessive soil moisture inhibits this fungus. (2) An acid soil reaction fails as a reliable control for all damping-off. Though acid soil will control damping-off by *Pythium*, it may stimulate that caused by *Rhizoctonia*. However, acid treatment of the soil may have a beneficial effect through partial disinfection of the soil surface. (3) Since the trend of postemergence damping-off is up with rising temperature and since this is the loss commonly seen by nurserymen, it has been concluded that damping-off is a warm-temperature disease. However, at low temperatures there may be excessive *Pythium* losses which go unobserved because they occur before emergence and are thus imputed to poor seed germination.

The present observations have been made under controlled conditions in the greenhouse. The temperature and moisture have been constant at the chosen levels. In nature these factors often fluctuate rapidly and widely. The possibilities are open that the responses of host and parasites might be different in fluctuating environments

from those in constant environments. The influence of temperature, moisture, and soil reaction on *Pythium* and *Rhizoctonia* damping-off in the nursery is considered in another paper (*17*).

## SUMMARY

*Pythium irregulare* and *Rhizoctonia solani*, the principal damping-off fungi in Wisconsin forest nurseries (*16*), the diseases they induce, and their host red pine (*Pinus resinosa*) were studied under controlled conditions for their responses to several important environmental factors.

Temperature influenced the rate as well as the amount of seedling emergence. In uninoculated containers germination was poor at 12° and 15° C., good at 18° and 33°, and excellent at from 21° to 30°. The subsequent growth of the seedlings was best between 15° and 30°.

The effect of temperature on damping-off by the two fungi was striking. *Pythium* killed over 90 percent of the seedlings at 12° C., only about half of them at 33°, and had intermediate effects between these temperatures. At 12° *Rhizoctonia* damped-off only a few seedlings. This loss increased to a maximum of approximately 58 percent at 24° to 30° and then declined at 33°.

The optimum temperature for growth of both fungi on potato-dextrose agar was 28° C. At 4° *Rhizoctonia* approached a minimum though *Pythium* continued to grow moderately well. Decline in growth rate above 28° was more rapid for *Pythium* than for *Rhizoctonia*. There appeared to be no relation between rate of growth by *Pythium* in cultures and the amount of damping-off in soil. However, the curve for damping-off by *Rhizoctonia* paralleled that for fungus growth.

With moisture content, *Pythium* damping-off increased from a minimum at 13 percent to a maximum at 100 percent. *Rhizoctonia* damping-off in the soil gradually increased from a high level at 13 percent to a maximum at 68 percent and then decreased to a minimum at saturation.

A successful method for controlling the moisture content of the surface one-half inch of soil, in which damping-off fungi are most active, has been described.

The root lengths of the red pine seedlings were greater in the relatively dry soils than in the wet soils.

Air humidity had little or no influence on *Pythium* damping-off, but high air humidity increased the loss caused by aerial mycelium of *Rhizoctonia*.

Acidity also was important to both mycelial and host growth. In culture, growth of *Pythium* extended from pH 3.7 to about 9 and was best between about 5 and 8. *Rhizoctonia* grew from pH 2.4 to about 9. Most rapid growth was between about 3.5 and 7.5 The optimum range for development of red pine was from about pH 4.7 to 6. Reactions below pH 4.7 and above 6 were relatively toxic.

With respect to damping-off, a broad range favorable for *Pythium* lay between pH 5.2 and 8.5 Below 5.2 the loss was comparatively small. A close relation appeared between the rate of growth in culture and the severity of *Pythium* damping-off. However, for *Rhizoctonia* the range from pH 5.2 to 7.8 was only moderately favorable,

and increase was rapid with acidity stronger than pH 5.2. Increase in damping-off by both fungi at levels above pH 7 appeared associated with decline in host development.

Damping-off by *Pythium* and *Rhizoctonia* was shown to be two distinct diseases.

Information about the influence of environment on the development of these diseases is helpful for accurate interpretation of disease development in the nursery.

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# INHERITANCE OF SYMPTOM EXPRESSION OF BEAN MOSAIC VIRUS 4 [1]

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## INTRODUCTION

Zaumeyer and Harter [2] [3] have recently described a new virus disease of beans (*Phaseolus vulgaris* L.) produced by bean mosaic virus 4 (southern bean mosaic virus 1). Eighty varieties or strains of beans were inoculated and all were found to be susceptible, but the expression of susceptibility varied with the variety used. Twenty-four varieties were homozygous for susceptibility to local lesions, 48 were homozygous for susceptibility to systemic infection, and 8 were heterozygous.

Homozygous varieties or strains that manifested local lesions, although susceptible to the virus at points of inoculation, were immune to the systemic infection. The virus could be recovered from the leaves showing local lesions but not from any other portion of the plant. Local lesions, if they occurred, appeared in 2 or 3 days after inoculation as somewhat circular, brownish-red, necrotic spots that ranged from 1 to 3 mm. in diameter (fig. 1, *A*, *C*). Leaves having numerous necrotic spots dropped off within a short time, thus eliminating the virus. Plants locally infected were susceptible to reinfection.

All the plants that did not show the local-lesion type of infection developed the systemic mottling (fig. 1, *B*, *D*) about 10 days after inoculation. In some varieties the mottling was mild, with little leaf malformation or distortion or plant stunting; in others the leaves were curled and reduced in size, and the plants were stunted. The yield of seed from such plants was much smaller than that from healthy plants. The virus was recovered from all portions of a systemically infected plant, and juice extracted from such a plant always produced necrotic lesions when inoculated to varieties susceptible to the local lesions. No variety was immune to the virus.

The studies reported herein deal with the inheritance of two types of response, as indicated by the segregation of $F_2$ and $F_3$ hybrids inoculated with bean mosaic virus 4. These two types of plant response to infection will be referred to throughout the paper as (1) local-lesion infection, in which only local lesions occur on inoculated leaves with no apparent injury to the plant and with no further development of the disease; and (2) systemic-mottle infection, in which mottling and general injury to the plant follow inoculation with a reduction in yield corresponding to the severity of the disease.

[1] Received for publication November 20, 1942.

[2] ZAUMEYER, W. J., and HARTER, L. L. A NEW VIRUS DISEASE OF BEAN. (Phytopath. Note) Phytopathology 32: 438-439. 1942.

[3] ZAUMEYER, W. J., and HARTER, L. L. TWO NEW VIRUS DISEASES OF BEANS. Jour. Agr. Res. 67: In press. 1943.

## MATERIAL AND METHODS

The virus used in these studies was isolated from infected beans growing under greenhouse conditions at Beltsville, Md.

The crosses were originally made under controlled greenhouse con-

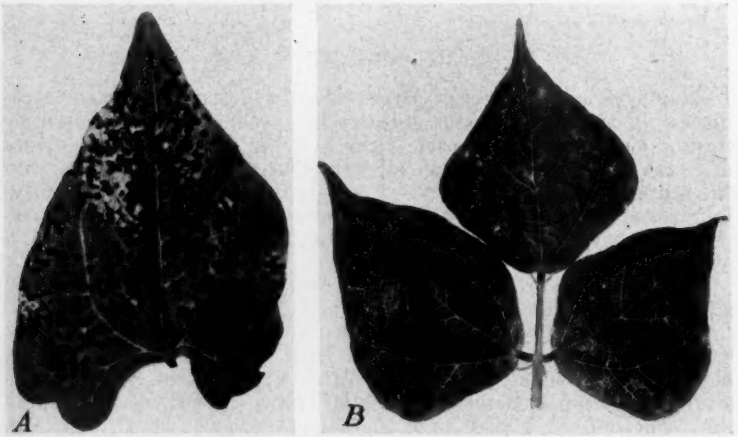





FIGURE 1.—Host reaction to bean mosaic virus 4: *A*, Local necrotic lesions on Pinto (Colorado strain); *B*, systemic mottle on No. 780–2H; *C*, local necrotic lesions on $F_2$ plant of a cross between Pinto (Colorado strain) and No. 780–2H; *D*, systemic mottle on another $F_2$ plant of same cross.

ditions for the study of the inheritance of resistance to another disease; but, when it was noted that one of the parents of each cross was subject only to the local-lesion type of symptoms produced by bean mosaic virus 4 (fig. 1, *A*, *C*), and the other parent showed only the systemic-mottle type of symptoms (fig. 1, *B*, *D*), it was decided to use

this material to determine the mode of inheritance of the expression of symptoms of bean mosaic virus 4. $F_1$ crosses were not tested. Two of the $F_2$ crosses were reciprocals of each other (see table 2) and the others were not, but all reacted in a similar manner.

The $F_2$ plants were inoculated in the greenhouse at Beltsville, Md. The seed was planted in 4-inch pots, and the plants were inoculated when the simple leaves were about three-fourths developed. Carborundum powder No. 38713 was dusted lightly on the leaves, and one simple leaf of each plant was inoculated. This was done in the usual manner by lightly rubbing the leaves with a cheesecloth pad dipped in a slightly diluted extract of the virus. After inoculation the leaves were washed with a fine spray of water. The records of plant response were made about 4 or 5 days after inoculation, and plants on which local lesions were formed and those on which they did not form were labeled and chosen at random for transplanting to greenhouse beds, where they were allowed to grow to maturity. Those that were not transplanted remained in the pots for at least 14 days in order that the number systemically infected might be determined. The number of transplanted plants systemically infected was also recorded. In cases where the mottle symptoms were in doubt, juice was extracted from a few leaves and inoculated to plants of the Ideal Market variety, which is very susceptible to the local-lesion infection. By this method proof was established of the presence or absence of the virus in such plants.

The seed harvested from transplanted $F_2$ plants was grown in 4-inch pots, and the $F_3$ plants produced were tested for their reaction to bean mosaic virus 4. In case of doubt as to whether certain $F_3$ plants were systemically infected, the same procedure was followed as with the $F_2$ inoculated plants.

All data have been subjected to the $\chi^2$ test for goodness of fit to certain theoretical ratios. This test was applied independently to $F_2$ families from individual $F_1$ plants, to $F_3$ families from $F_2$ plants, and to the totals of a number of tested families.

## EXPERIMENTAL RESULTS

### REACTION OF SELFED PARENTS

The reaction to bean mosaic virus 4 of the selfed parents used in the several crosses is shown in table 1. In every case a variety or strain showed either the local-lesion infection or the systemic infection, but never both. Hundreds of plants of commercial varieties were inoculated, and all were found to be susceptible to either local or systemic infection.

TABLE 1.—*Reaction of the selfed parents used in the various crosses when inoculated with bean mosaic virus* [4]

| Parent | Plants inoculated | Plants showing— | |
|---|---|---|---|
| | | Local-lesion infection | Systemic-mottle infection |
| | *Number* | *Number* | *Number* |
| Blue Lake | 30 | 30 | 0 |
| Brittle Wax | 30 | 0 | 30 |
| Corbett Refugee (No. 7–B–2)[1] | 30 | 30 | 0 |
| Cranberry | 30 | 0 | 30 |
| Pinto (Colorado strain) | 30 | 30 | 0 |
| Pinto (Idaho strain) | 30 | 30 | 0 |
| Red Kidney | 30 | 0 | 30 |
| No. 765 [1 2] | 30 | 30 | 0 |
| No. 780–2H [1 3] | 30 | 0 | 30 |

[1] Numbers carried in the files of the writers.
[2] A selection from the Kentucky Wonder Wax variety.
[3] A white-seeded Kentucky Wonder hybrid.

### REACTION OF $F_2$ GENERATION

The $F_1$ plants were not tested. An analysis of the $F_2$ data (table 2) shows that the local-lesion type of infection (fig. 1, *A*, *C*) is dominant to the systemic-mottle type (fig. 1, *B*, *D*) with a 3:1 segregation, indicating a single-factor difference. The reciprocals behaved alike. The $\chi^2$ values indicated that the observed data fit the calculated quite closely except in 1 case where the $\chi^2$ value exceeded the 5-percent but not the 1-percent point. Out of a total of 450 plants, 335 showed local-necrotic infection and 115 systemic infection. This segregation is a close fit to a 3:1 ratio, with a $\chi^2$ value of 0.075.[4]

TABLE 2.—*Reaction of $F_2$ progenies when inoculated with bean mosaic virus* [4]

| Percentage of cross | Plants inoculated | Plants showing— | | $\chi^2$ for 3 : 1 ratio [1] |
|---|---|---|---|---|
| | | Local-lesion infection | Systemic-mottle infection | |
| | *Number* | *Number* | *Number* | |
| Blue Lake × No. 780–2H [2] | 83 | 66 | 17 | 0.904 |
| No. 780–2H [2] × Blue Lake | 34 | 25 | 9 | .038 |
| Brittle Wax × No. 765 [2] | 147 | 109 | 38 | .056 |
| Cranberry × Pinto (Idaho strain) | 37 | 27 | 10 | .093 |
| Pinto (Colorado strain) × No. 780–2H [2] | 65 | 41 | 24 | 4.928 |
| Red Kidney × Corbett Refugee (No. 7–B–2) [2] | 84 | 67 | 17 | .904 |

[1] 5-percent point=3.841.
[2] Numbers carried in the files of the writers.

### REACTION OF $F_3$ GENERATION

Of 63 $F_3$ families derived from $F_2$ plants that showed the local-lesion type of infection, 24 were homozygous for the local-lesion type of infection and 39 were heterozygous, when inoculated with bean mosaic virus 4 (table 3). The deviation from the calculated 1:ratio of homozygous to heterozygous local-lesion type families was non-significant, with a $\chi^2$ value of 0.589.[5] In the 39 heterozygous

[4] 5-percent point=3.841.
5-percent point=3.841.

families, the segregation of plants with the local-lesion type of infection and plants with the systemic-mottle type showed a good fit to a 3 : 1 ratio (table 3). The progenies from 29 $F_2$ selfed families of the systemic-mottle type of infection were homozygous for systemic infection (table 3). These results contributed additional proof in support of the single-factor hypothesis.

TABLE 3.—*Reaction of $F_3$ progenies descended from previously tested $F_2$ progenies when inoculated with bean mosaic virus 4*

| Classification in $F_3$ generation | Families tested [1] | Plants | Plants showing— | |
|---|---|---|---|---|
| | | | Local-lesion infection | Systemic mottle infection |
| | *Number* | *Number* | *Number* | *Number* |
| Homozygous local-lesion type | 24 | 1,065 | 1,065 | 0 |
| Heterozygous local-lesion type : | | | | |
| Observed | 39 | 1,494 | 1,152 | [2] 342 |
| Calculated 3 : 1 ratio | | | 1,120.5 | 373.5 |
| Homozygous systemic-mottle type | 29 | 424 | 0 | 424 |

[1] $\chi^2$ for 1 : 2 distribution of families = 0.589; 5-percent point = 3.841.
[2] $\chi^2$ for total population was 3.541; 5-percent point = 3.841. Accumulated $\chi^2$ was 62.547; 5-percent point = 62.592 for 39 degrees of freedom.

## DISCUSSION

The results clearly demonstrate that in the hybrids investigated the inheritance of the expression of symptoms of bean mosaic virus 4 is governed by a single genetic-factor difference and that the local-lesion infection is dominant to the systemic-mottle type. The reaction of the heterozygous plants was indistinguishable from that of plants homozygous for the local-lesion type of infection.

Holmes,[6] in studying the inheritance of the localization of tobacco mosaic virus in certain pepper varieties, found responses similar to those reported here for beans. His results indicated that, in crosses between pepper varieties that showed only necrotic lesions and varieties that were systemically infected, a single dominant Mendelian factor governed the inheritance of the local-lesion type of infection caused by tobacco mosaic virus. He reported similar results in investigations dealing with the inheritance of resistance to tobacco mosaic in tobacco[7] and *Browallia*.[8]

In the tests here reported a few plants in certain progenies showed the heritable leaf variegation described earlier.[9] The greater the variegation the less chlorophyll the leaves contained. In most instances variegated plants of the dominant genotype did not respond to the local-lesion infection, and, when they did, the lesions were smaller than those on normal plants. On the other hand, the virus was not prevented from becoming systemic in the variegated plants of the recessive genotype. Although the mottled symptoms were difficult to diagnose because of the variegated character, juice from such plants

[6] HOLMES, F. O. INHERITANCE OF ABILITY TO LOCALIZE TOBACCO-MOSAIC VIRUS. Phytopathology 24 : 984–1002, illus. 1934.
[7] ——— INHERITANCE OF RESISTANCE TO TOBACCO-MOSAIC DISEASE IN TOBACCO. Phytopathology 28 : 553–561, illus. 1938.
[8] ——— INHERITANCE OF RESISTANCE TO TOBACCO-MOSAIC DISEASE IN BROWALLIA. Phytopathology 28: 363–369, illus. 1938.
[9] ZAUMEYER, W. J. INHERITANCE OF A LEAF VARIEGATION IN BEANS. Jour. Agr. Res. 64: 119–127, illus. 1942.

produced local lesions on Ideal Market, proving the presence of the virus. It is not known why local infection was inhibited in the variegated plants and systemic infection was not.

Immunity to bean mosaic virus 4 has not been observed in any variety thus far tested. Thirty-two varieties have been found that possess the dominant character for virus localization. Twenty-four of these are homozygous for this character and hence are immune to the systemic type of infection. These varieties can be classified as commercially resistant, since little or no damage to the plants results from such infection. Unfortunately most of the important canning and market-garden varieties carry the recessive factor and are therefore subject to the systemic type of infection. Since the inheritance of the types of symptom expression is of a simple nature, little difficulty should be experienced in producing through hybridization varieties susceptible only to local-lesion infection. It would seem that the production of such varieties could eventually eliminate the loss caused by bean mosaic virus 4 in beans. Such varieties are being developed at the present time.

## SUMMARY

The inheritance of the expression of symptoms of bean mosaic virus 4 was found to be governed by a single allelomorphic pair of Mendelian factors. Plants carrying the dominant allelomorph are susceptible to a local-lesion type of infection that causes little or no damage, whereas the homozygous recessive plants are susceptible to a systemic type of infection that causes leaf mottling, stunting, and reduction of yield.

Although immunity to bean mosaic virus 4 has not been observed, varieties possessing the dominant gene for virus localization are considered commercially resistant, since in these varieties the infection does not become systemic. Such varieties are being used in hybridization as parental material for producing other commercially resistant varieties.

# THE OCCURRENCE OF CITRIC AND ISOCITRIC ACID IN BLACKBERRIES AND IN DEWBERRY HYBRIDS[1]

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## INTRODUCTION

Isocitric acid was first found in nature as the principal acid in eastern blackberries (*9*, *11*).[3] Blackberries from the State of Washington were also found to contain isocitric acid (*2*). On the other hand, the principal acid of the Loganberry was found to be citric acid (*8*, *10*). These investigations have now been extended to include three named varieties of blackberries, the Brainerd, Crandall (Macatawa), and Texas Wonder, and the dewberry hybrids, Young (Youngberry) and Boysen (Boysenberry).

## EXPERIMENTAL METHODS AND MATERIAL

Since there is no published method for the determination of isocitric acid, the ester-distillation method (*3*, *4*, *5*, *6*, *7*, *9*, *11*) which led to the discovery of isocitric acid in blackberries, was used. As this general procedure for the separation of the nonvolatile acids and the fractionation of their ethyl esters has been described previously many details will be omitted here. In this procedure the nonvolatile acids, such as citric, isocitric, and malic, are converted into the ethyl esters, the latter are fractionated, and hydrazides are prepared from the various fractions. The individual acids are identified by the boiling point and optical rotation of their esters, and by the rate and manner of crystallization, the melting point, and the crystalline form of the hydrazides. From the quantities of the various esters obtained, approximate values for the amounts of the corresponding acids present may be calculated.

The fruit used in the investigation was obtained by the Fruit and Vegetable Chemistry Laboratory of the Bureau of Agricultural and Industrial Chemistry in Los Angeles and shipped frozen to Washington, D. C. Table 1 shows the varieties used, the quantities, and the yield of crude ethyl esters.

TABLE 1.—*Fruits examined, quantities used, and yields of crude ethyl esters*

| Variety | Quantity of fruit | Crude ethyl esters |
|---|---|---|
| | *Grams* | *Grams* |
| Brainerd | 4,760 | 52.8 |
| Crandall | 8,200 | 67.5 |
| Texas Wonder | 7,860 | 60.0 |
| Young | 6,600 | 65.4 |
| Boysen | 7,060 | 58.5 |

[1] Received for publication October 22, 1942.
[2] Died November 9, 1940.
[3] Italic numbers in parentheses refer to Literature Cited, p., 295.

Table 2 gives the results of the experiments. All samples were fractionated at 10 mm. Optical crystallographic examinations were made by G. L. Keenan, Food and Drug Administration, Federal Security Agency.

## EXPERIMENTAL RESULTS

TABLE 2.—*Physical constants of the fractions of the ethyl esters of designated blackberries and dewberries*

| Fraction | Boiling point | Weight | Optical rotation at 20° C. | Melting point of hydrazide | Mixed melting point with known hydrazide |
|---|---|---|---|---|---|
| **BRAINERD** | | | | | |
| | °C. | *Grams* | | °C. | °C. |
| 1 | 84–123 | 1.8 | | 178 | 178 *l*-malic hydrazide. |
| 2 | 123–125 | 12.9 | −11.7 | 178 | 178 *l*-malic hydrazide. |
| 3 [1] | 175–177 | 27.0 | [2] +5.3 | 176 | |
| **CRANDALL (MACATAWA)** | | | | | |
| 1 | 112–126 | 0.9 | | 178 | 178 *l*-malic hydrazide. |
| 2 | 126–130 | 8.0 | −11.4 | 178 | |
| 3 [1] | 180–186 | 51.6 | [2] +5.1 | 176 | |
| **TEXAS WONDER** | | | | | |
| 1 | 114–125 | 10.1 | −12.0 | 179 | 179 *l*-malic hydrazide. |
| 2 | 125–165 | 1.6 | | 179 | |
| 3 | 173–176 | 3.2 | | 168 | 167 isocitric hydrazide. |
| [1] | 176–181 | 32.9 | [2] −1.2 | 172 | |
| **YOUNG** | | | | | |
| 1 | 122–137 | 4.1 | −11.0 | 179 | 180 *l*-malic hydrazide. |
| 2 | 137–170 | 1.5 | | 177 | 178 *l*-malic hydrazide. |
| 3 [3] | 170–171 | 46.5 | | 106 | |
| 4 [3] | 171–181 | 4.6 | [2] −2.0 | 173 | 174 isocitric hydrazide. |
| **BOYSEN** | | | | | |
| 1 | Below 118 | 1.0 | | 178 | 178 *l*-malic hydrazide. |
| 2 | 120–130 | 4.1 | −11.3 | 178 | 178 *l*-malic hydrazide. |
| 3 [3] | 170–175 | 42.4 | | 105 | |
| 4 [3] | Above 175 | 1.3 | | 172 | 172 isocitric hydrazide. |

[1] Optical crystallographic examination confirmed the identification of the hydrazide as isocitric hydrazide.
[2] The variable values shown are probably due to the formation of a mixture of triethyl isocitrate and diethyl isocitrate lactone from isocitric acid during esterification.
[3] Optical crystallographic examination confirmed the identification of the hydrazides of fractions 3 and 4 as citric and isocitric hydrazides, respectively.

The juice of the Brainerd blackberries was examined for citric acid by the Association of Official Agricultural Chemists' method (*1*).

TABLE 3.—*Proportions of isocitric, citric, and* l*-malic acid found in berries examined*

| Variety | Acids | | |
|---|---|---|---|
| | Isocitric | Citric | *l*-malic |
| | *Percent* | *Percent* | *Percent* |
| Brainerd | 65 | 0 | 35 |
| Crandall | 85 | 0 | 15 |
| Texas Wonder | 75 | 0 | 25 |
| Boysen | 4 | 85 | 11 |
| Young | 6 | 86 | 8 |

The result (0.05 percent) was practically negative, indicating not more than a trace of citric acid.

The approximate proportions of the isocitric, citric, and *l*-malic acids are given in table 3.

Isocitric acid predominates in the Brainerd, Crandall, and Texas Wonder varieties, all of which are eastern blackberries. Citric acid is the principal acid of the Boysen and Young, and it also occurs almost exclusively in the Logan (Loganberry), all three of which are trailing varieties related to the Pacific coast blackberry. From the above it appears that there may be a relationship between the type of berry and the principal acid (isocitric or citric). No final conclusion should be drawn, however, until a larger number of varieties of both bush and trailing blackberries have been examined.

## SUMMARY

The nonvolatile acids present in three blackberries, Brainerd, Crandall, and Texas Wonder, and in two trailing hybrids, the Boysen and the Young, have been investigated by the ester distillation method. The predominating acid in the three blackberries was found to be isocitric acid, whereas in the Boysen and Young dewberries it is citric acid.

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